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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Nicolaides *et al.*

Serial No.: 09/558,149 **Group Art Unit:** 1632

Filing Date: April 26, 2000 **Examiner:** R. Shukla

For: METHODS FOR GENERATING HYPERMUTABLE ORGANISMS

Assistant Commissioner for Patents
Washington D.C. 20231

Considered 6/2/03 RDS

Supplemental Declaration under 37 CFR 1.131

I, J. Bradford Kline, hereby state the following:

1. This Supplemental Declaration is provided to supplement my Declaration provided in the above-referenced case on September 26, 2002.

2. The transgenic mice that were generated comprising the PMS2-134 truncation mutant mismatch repair gene were examined for defects in mismatch repair resulting in hypermutability according to the following method of examining microsatellite instability (a hallmark of hypermutability):

Microsatellite Instability in the Morphomouse Genome

Wildtype or morphomouse genomic DNA was purified from the liver and diluted to 1pg/µl. 1 pg (0.5 haploid genome equivalents) was amplified by polymerase chain reaction (PCR) in a total volume of 12.5 µl using primers specific for the microsatellite marker mBat-37 using the following reaction conditions:

Touchdown cycle -
95 5min
94 1min 60 1min 72 1min
94 1min 59 1min 72 1min
94 1min 58 1min 72 1min
94 1min 57 1min 72 1min
94 1min 56 1min 72 1min
94 1min 55 1min 72 1min
94 1min 54 1min 72 1min

94 1min 53 1min 72 1min
94 1min 52 1min 72 1min 30x cycles
72 10min

After amplification, 100ul of dH_2O was added to the PCR reaction, 1ul of which is used for fragment analysis on the Beckman CEQ2000. Peaks were called using CEQ8000 Fragment Analysis software.

3. The data in the attached Figure shows an example of a morphomouse showing mutations in its DNA in the mBat-37 locus.

4. For morphomouse 685.4, among the 31 single clones examined from testes DNA, four (4) clones showed deviation from the wild-type poly-A microsatellite: Two (2) clones showed additional nucleotides at this locus (expansion), and two (2) showed a decrease in the number of nucleotides at the locus (contraction), for a mutation rate of 12.9%.

6. All statements made herein of my own knowledge are true, and all statements made herein on information and belief are believed to be true.

7. I hereby acknowledge that willful false statements and the like are punishable by fine or imprisonment, or both under 18 U.S.C. §1001, and may jeopardize the validity of the application or any patent issuing thereon.

J. Bradford Kline

4-17-03

Date

Microsatellite Instability in F1 Testes of 685 Line